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GC-MS analysis, antioxidant, antibacterial and anticancer activities of methanol bark extract of *Albizia lebbeck* (L.)

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ABSTRACT

Albizia lebbeck is a deciduous tree with compound leaves, flat oblong fruits, round cream seeds are colored and wild growth. *Albizia lebbeck* is found throughout India, Bangladesh, tropical and subtropical Asia and Africa. For toothache and gum diseases, bark parts are essentially used. Extracted residues of the leaves and barks are protective against bronchial asthma and other allergic disorders. Barks and seeds are astringent and are used in the treatment of piles and diarrhoea. Current research studies were carried out for evaluating the antioxidant, antibacterial and anticancer potentiality of methanol bark extract of *A. lebbeck* and to identify the bioactive compounds by GC-MS analysis. Anticancer activity was carried out by MTT reduction assay method on MCF 7 cancer cell line. Antioxidant activities such as DPPH˙ radical, $ABTS^{\bullet+}$ radical cation, superoxide ($O_2^{\cdot-}$) radical, phosphomolybdenum reduction and Fe^{3+} reducing power assays were carried out for bark extract. The maximum DPPH˙ radical scavenging activity was 90.97±0.36% at 120 µg/mL concentration and its IC_{50} was 35.89 µg/mL concentration. The maximum $ABTS^{\bullet+}$ radical cation scavenging activity was 95.65±0.30% at 30 µg/mL concentration and its IC_{50} was 13.79 µg/mL concentration. The maximum superoxide ($O_2^{\cdot-}$) radical scavenging activity was 56.45±0.35% at 60 µg/mL concentration and its IC_{50} was 46.28 µg/mL concentration. The maximum phosphomolybdenum reduction showed 88.98±0.28% at 120 µg/mL concentration and Fe^{3+} reduction showed 80.55±0.34% at 120 µg/mL concentration. The RC_{50} of Mo^{6+} reduction and Fe^{3+} reduction were 31.39 µg/mL and 39.16 µg/mL concentration respectively. The maximum MCF 7 cell death was 79.36±0.23% at 250 µg/mL concentration and the IC_{50} was 47.12 µg/mL concentration.

Keywords: *Albizia lebbeck*, DPPH˙ radical, MTT assay, antibacterial, anticancer, GC-MS.

INTRODUCTION

Albizia lebbeck commonly known as Siris belonging to the family Fabaceae, has been broadly used in indigenous systems of Indian medicine due to its numerous therapeutic properties. The genus *Albizia* (Fabaceae) comprises approximately 150 species, mostly trees and shrubs native to tropical and subtropical regions of Asia and Africa [1]. *A. lebbeck* is indigenous to the Indian subcontinent and to those areas of South-East Asia with a marked dry season, such as north-eastern Thailand, parts of Malaysia and in the eastern islands of Indonesia [2].

A. lebbeck is a deciduous tree, it can grow to a height of 30 m and it contains 1m diameter of the stem, but it is commonly at a maturity stage will grow 15-20 m tall and 50 cm in diameter. Generally, the bark of the tree is the gray fissured corky bark, somewhat flaky and the inner bark reddish colour. It is multi-stemmed when grown in the open but can produce a single straight stem when it grown in plantations [3, 4]. The compound leaves are bipinnate, glabrous or slightly hairy on the axis, pinnae in 2-4 pairs, each with 2-11 pairs of obliquely oblong to elliptic-oblong leaflets, 15-65 x 5-35 mm, shortly stalked, initially bright green and maturing to a duller glaucous green and folding at night [5]. It is fully deciduous but for only a brief period (4-6 weeks) in the dry season. The glabrous glands are raised, elliptic to circular, on the upper side of the stalk, close to the base and between most pairs of leaflets [6]. The inflorescence consists of large clusters 5-7.5 cm wide of fragrant pedunculate globular flower heads, 15-40, on stalks 5-10 cm long. The corolla is 5.5-9 mm long, glabrous, cream, white or green, with numerous pale green stamens with filaments 15-30 mm long (Figure 1). The entire inflorescence is 'fluffy' in appearance, 60 mm in diameter, yellow-green with a pleasant fragrance. The pods are pale straw to light brown at maturity, narrow-oblong, 12-35 x 3-6 cm, papery-leathery, swollen over the seeds and not constricted between them, indehiscent and borne in large numbers. Seeds are brown, flat, orbicular or elliptic, 8-10 x 6-7 mm, transversely placed with 3-12 in each pod.

Taxonomic Classification

Domain: Eukaryota
Kingdom: Plantae
Phylum: Tracheophyta
Class: Magnoliopsida
Order: Fabales
Family: Fabaceae
Genus: *Albizia*
Species: *Albizia lebbeck* (L.)

Figure 1: Leaves and barks of *Albizia lebbeck*

EXPERIMENTAL METHODOLOGY

Sample Collection and Condensation process

The samples were barks of *A. lebbeck* which were collected from Avudaiyarkovil, Pudhukkottai, Tamilnadu, India. The barks were cut into small pieces and immersed in methanol for 72 h. The plant residue was filtered and condensed by using rotary evaporator at 50°C which yields red gummy extract.

Qualitative phytochemical analysis

The methanol bark extract of *A. lebbeck* was subjected to preliminary phytochemical analysis for different classes of phytoconstituents using specific standard reagents [7, 8].

Phenols content analysis

Folin-Ciocalteau reagent method was preferred to estimate phenolic compounds [9] with slight modifications. One hundred µL of methanol bark extract of *A. lebbeck* was added with 900 µL of distilled water and 1 mL of Folin-Ciocalteau reagent (1:10 diluted with distilled water). 1 mL of aqueous solution of Na_2CO_3 (20% w/v) was mixed after 5 mins. The mixture was kept undisturbed for 30 min incubation in dark condition. The absorbance was measured by UV-Vis spectrophotometer at 765 nm. The total phenolic content was expressed in terms of gallic acid equivalent (µg/mg of extract), which is a common reference compound.

Flavonoids content analysis

Aluminium chloride method was used to determine the total flavonoid content of methanol bark extract of *A. Lebbeck* with slight modification as described [10]. Five hundred µL of extract was added with 500 µL of methanol and 500 µL of (5% w/v) sodium nitrite solution followed by 500 µL of (10% w/v) aluminium chloride solution. The mixture was shaken well and 1 mL of 1 M NaOH solution was added and incubated at room temperature for 30 min. Absorbance was measured at 510 nm using spectrophotometer and the result was expressed as (µg/mg of extract) quercetin equivalent.

***In vitro* antioxidant activities**

DPPH˙ radical scavenging activity

The antioxidant activity of methanol bark extract of *A. lebbeck* was measured by DPPH free radical scavenging method [11]. One mL of 0.1 mM DPPH solution in methanol was combined with 1 mL of various concentrations (20-120 µg/mL) of bark extract. The mixture was then allowed to stand for 30 min incubation in dark. Distilled water was used as the reference standard. One mL methanol was combined with 1 mL DPPH solution was used as the control. The decrease in absorbance was measured using UV-Vis Spectrophotometer at 517 nm. Ascorbic acid was used as standard reference. The percentage of inhibition was calculated as:

$$\% \text{ of DPPH}^{\cdot} \text{ radical inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}}\right] * 100$$

ABTS$^{\bullet+}$ radical cation scavenging activity

The antioxidant capacity was determined in terms of the ABTS$^{\bullet+}$ radical cation scavenging activity following the procedure described [12]. ABTS$^{\bullet+}$ was obtained by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate and the mixture was left undisturbed in the dark at room temperature for 12-16 h before use. The ABTS solution (stable for 2 days) was diluted with distilled water to reach an absorbance of 0.70±0.02 at 734 nm. Various concentrations of methanol bark extract of *A. lebbeck* (5-30 µg/mL) mixed with 500 µL of dilute ABTS$^{\bullet+}$ radical cation solution and the absorbance was measured after 10 min. Ascorbic acid was used as standard reference. The percentage of inhibition was calculated as:

$$\% \text{ of ABTS}^{\bullet+} \text{ radical cation inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}}\right] * 100$$

Superoxide ($O_2^{\cdot-}$) radical scavenging activity

Superoxide ($O_2^{\cdot-}$) radical scavenging activity was carried out by the method [13]. The reaction contained with different concentrations (10-60 µg/mL) of methanol bark extract of *A. lebbeck*, 50 mM of phosphate buffer (pH 7.6), 1.5 mM of riboflavin, 12 mM of EDTA and 50 mM of NBT, added in that sequence. The reaction was started by illuminating the mixture for 15 min. After illumination, the absorbance was measured at 590 nm and IC_{50} was calculated. Ascorbic acid was used as standard reference. The percentage of inhibition was calculated as:

$$\% \text{ of superoxide } (O_2^{\cdot-}) \text{ radical inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}}\right] * 100$$

Phosphomolybdenum reduction activity

The antioxidant capacity of methanol bark extract of *A. lebbeck* was performed as described [14]. The bark extract with concentrations ranging from 20 - 120 µg/mL was mixed with 1 mL of reagent solution containing ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulphuric acid (600 mM). The test tubes containing reagent solution was incubated in water bath at 95°C for 90 min. The absorbance of the coloured complex was measured at 695 nm. Ascorbic acid was used as standard reference. The percentage of reduction was calculated as:

$$\% \text{ of phosphomolybdenum reduction} = \left[\frac{\text{Sample} - \text{Control}}{\text{Sample}}\right] * 100$$

Ferric (Fe^{3+}) reducing power activity

The reducing power of methanol bark extract of *A.lebbeck* was determined by slightly modified method [15]. One mL of bark extract of different concentrations (20 - 120 µg/mL) was combined with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1% w/v). The mixtures were then incubated at 50°C for 30 min in water bath. One mL of trichloroacetic acid (10% w/v) was added to each mixture and shaken well. Then to the mixture 1 mL of freshly prepared $FeCl_3$ (0.1% w/v) was added and the absorbance was measured at 700 nm using Spectrophotometer. Ascorbic acid was used as standard reference. The percentage of reduction was calculated as:

$$\% \text{ of } Fe^{3+} \text{ reduction} = \left[\frac{\text{Sample} - \text{Control}}{\text{Sample}}\right] * 100$$

Antibacterial activity by agar well diffusion method

Microbial strains

The microorganisms such as *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris* and *Shigella flexneri* were used for the evaluation of antimicrobial activity.

Aseptic conditions

The aseptic chamber which consist of a wooden box (1.3m x 1.6m x 0.6m) with a door, was cleaned with 70% ethanol and irradiated with short wave UV light (from lamp).

Nutrient broth agar medium

Nutrient broth agar medium was prepared (peptone-5 g; yeast extract-3 g; NaCl-5 g; distilled water-100 mL; pH-7±0.2; agar-20 g) according to the standard methods and was suspended in 200 mL of distilled water in a 500 mL conical flask, stirred, boiled to dissolve and then autoclaved at 15 lbs and at 121ºC for 15 minutes. The hot medium was poured in sterile petri-plates and could solidify for 15 min in the aseptic laminar chamber [16].

Agar well diffusion method

Determination of antimicrobial potential of the methanol bark extract of *A. lebbeck* was carried out using the agar well diffusion method. The solidified nutrient agar in the petri-plates was inoculated by dispensing the inoculum using sterilized cotton swabs and spread evenly onto the solidified agar medium. Five wells were created in each plate with the help of a sterile well-borer of 8 mm diameter. The extract and standard were then poured into each well of desirable concentrations. Tetracycline was used as the standard with the concentration of 25 µg. All the plates containing extract loaded wells were incubated for 24 h at room temperature. After the incubation period, zone of inhibition were measured by calculating the diameter of zone of inhibition [17].

Thin layer chromatography

Thin layer chromatography (TLC) was carried out for the methanol bark extract of *A. lebbeck* in Merck TLC aluminium sheets, silica gel 60 F254 (20 x 20 cm), preloaded plates. The extract was spotted at 0.3 mm above from the bottom of the TLC plate. The chromatogram was developed in a mixture of suitable solvent system. The spots were visualized under UV light at 254 nm. The R_f values of the coloured spots were recorded [18] and calculated.

$$R_f \text{ value} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Anticancer activity of methanol bark extract of *A. lebbeck* on MCF 7 (Human breast cancer) cell lines

MTT assay method

Cell viability was measured with the conventional MTT reduction assay method as described with slight modifications [19]. Briefly, MCF 7 cells were seeded at the density of 5×10^3 cells/well in 96-well plates for 24 h, in 200 µL of RPMI (v/v) with 10% FBS (v/v). Then the culture supernatant was removed and RPMI containing various concentrations (0.781-250 µg/mL) of methanol bark extract of *A. lebbeck* was added and incubated for 48 h. After treatment the cells were incubated with 10 µL of MTT (5 mg/mL) at 37°C for 4 h and then with DMSO at room temperature for 1 h. The plates were read at 595 nm on a scanning multi-well spectrophotometer.

Gas Chromatography–Mass Spectrometry analysis

For GC-MS analysis, the methanol bark extract of *A. lebbeck* were injected into a HP-5 column (30 m X 0.25 mm i.d with 0.25 µm film thickness), Agilent technologies 6890 N JEOL GC Mate II GC-MS model. Following chromatographic conditions were used: Helium as carrier gas, flow rate of 1 mL/min; and the injector was operated at 200°C and column oven temperature was programmed as 50-250°C at a rate of 10°C/min injection mode. Following MS conditions were used: ionization voltage of 70 eV; ion source temperature of 250°C; interface temperature of 250°C; mass range of 50-600 mass units [20].

Identification of components

The database of National Institute Standard and Technology (NIST) having more than 62,000 patterns was used for the interpretation on mass spectrum of GC-MS. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical analysis of methanol bark extract (Table 1) of *A. Lebbeck* showed the presence of alkaloids, terpenoids, phenolic compounds, flavonoids, glycosides and saponins.

Table 1: Phytochemical screening of methanol bark extract of *A. lebbeck*

S. No	Phytochemicals	Tests	Results
1	Alkaloids	a. Mayer's reagent	+
		b. Picric acid test	+
2	Terpenoids	$CHCl_3$ + conc. H_2SO_4	+
3	Flavanoids	NaOH solution	+
4	Phenols	$FeCl_3$ solution	+
5	Glycosides	Sodium nitroprusside solution + Con. H_2SO_4	+
6	Saponins	Foam test	+

Determination of Total phenols and flavonoids content

Total phenols and flavonoids were quantified in the methanol bark extract of *A. lebbeck* showed to be responsible for the antioxidant activity. Phenols comprising of phenolic acids, flavonoids, biflavonoids, anthocyanins and isoflavonoids possess a wide spectrum of biochemical activities such as antioxidant, antimutagenic, anticarcinogenic, as well as ability for altering the expression of genes. Phenolic compounds characterized by hydroxylated aromatic rings are available in plants. Simple phenolics and flavonoids are important

phytochemicals. These compounds show a wide range of antioxidant activities and are dominantly effective against cancer and cardiac diseases. The antioxidant ability of phenolic compounds is determined by structure, the ease with which a hydrogen atom from an aromatic hydroxyl group can be donated to the free radicals [21].

The total phenol content was 380.3±0.42 µg/mg of GAE and total flavonoid content was 30.33±0.45 µg/mg of QE in the extract. The above results provide elaborative description of the antioxidant activity of bark extract of *A. lebbeck* with respect to their phenols and flavonoids content.

In Vitro antioxidant activities

DPPH˙ radical scavenging activity

The capability of methanol bark extract of *A. lebbeck* to neutralize free radicals formed was evaluated by 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). The maximum DPPH˙ radical scavenging activity was 90.97% at 120 µg/mL concentration and the IC_{50} was 35.89 µg/mL concentration (Table 2 and Figure 2). Bark extract of *A. lebbeck* demonstrated high capacity for scavenging free radicals by reducing the stable DPPH (1,1-diphenyl-2- picrylhydrazyl) radical to the yellow coloured 1,1-diphenyl-2-picrylhydrazine and the reducing capacity increased with increasing concentration of the extract. It was compared with the standard (IC_{50} = 2.88 µg/mL concentration) ascorbic acid.

Table 2: DPPH˙ radical scavenging activity of methanol bark extract of *A. lebbeck*

S. No	Concentration (µg/mL)	% of inhibition*
1	20	35.02±0.45
2	40	55.72±0.20
3	60	68.72 ±0.31
4	80	81.93±0.19
5	100	89.43±0.26
6	120	90.97±0.36

(*Average value of 3 replicates)

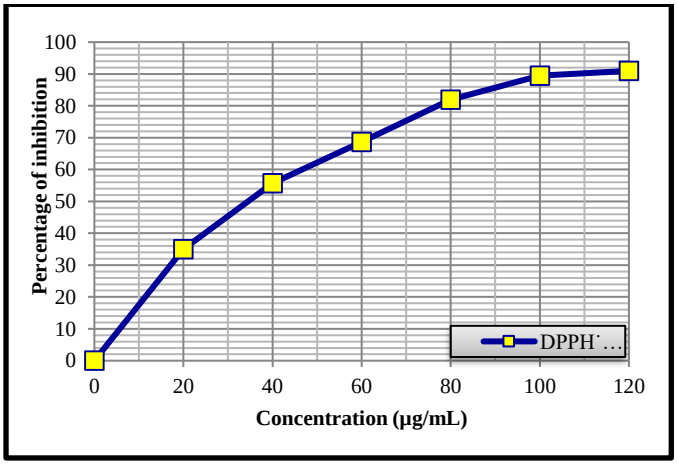


Figure 2: DPPH˙ radical scavenging activity of methanol bark extract of *A. lebbeck*

ABTS$^{\bullet+}$ radical cation scavenging activity

ABTS$^{\bullet+}$ is a blue colour produced by the reaction between ABTS and potassium persulfate and in the presence of the bark extract or ascorbic acid, preformed cation radical gets reduced and the remaining radical cation concentration was then quantified. The maximum ABTS$^{\bullet+}$ radical cation scavenging activity was 95.65% at 30 µg/mL concentration (Table 3 and Figure 3). The experiment demonstrated high antioxidant activity the IC_{50} of 13.79 µg/mL concentration and was compared with standard ascorbic acid (IC_{50} = 3.91 µg/mL concentration).

Table 3: ABTS$^{\bullet+}$ radical cation scavenging activity of methanol bark extract of *A. lebbeck*

S. No	Concentration (µg/mL)	% of inhibition*
1	5	19.57±0.29
2	10	39.13±0.44
3	15	54.35±0.10
4	20	65.22±0.38
5	25	73.91±0.17
6	30	95.65±0.30

(*Average value of 3 replicates)

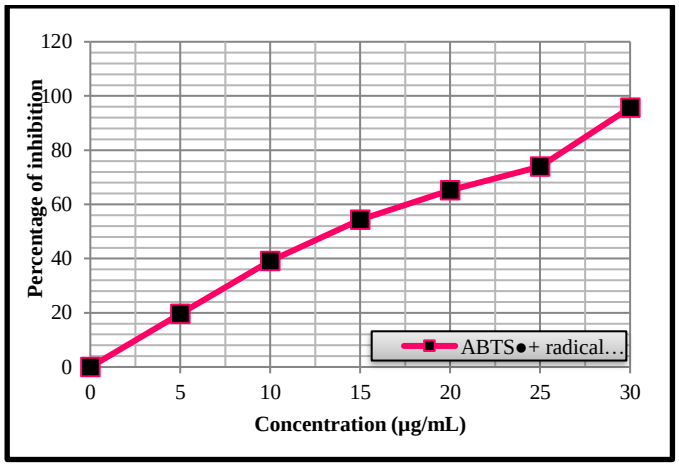


Figure 3: ABTS$^{\bullet+}$ radical cation scavenging activity of methanol bark extract of *A. lebbeck*

Superoxide ($O_2^{\cdot-}$) radical scavenging activity

Superoxide radical scavenging activity depends upon the ability of the extract to prevent blue formazan formation by neutralizing the superoxide radicals generated in riboflavin-light-NBT system. Superoxide is an oxygen-centered radical with selective reactivity. Although it is a relatively weak oxidant, superoxide exhibits limited chemical reactivity but can generate more dangerous species, including singlet oxygen and hydroxyl radicals, which cause the peroxidation of lipids. Superoxide can also reduce certain iron complexes such as cytochrome C. Superoxide anions are thus precursors to active free radicals that have potential for reacting with biological macromolecules and thereby inducing tissue damage. Antioxidant properties of some flavonoids are effective mainly via scavenging of superoxide anion radical [22, 23]. Superoxide anions derived from dissolved oxygen by the riboflavin-light-NBT system will reduce NBT in this system. In this method, superoxide anion reduces the yellow dye (NBT^{2+}) to produce the formazan, which is measured spectrophotometrically at 590 nm. Antioxidants can inhibit the blue NBT formation. The decrease of absorbance with antioxidants indicates the consumption of superoxide anion in the reaction mixture. The maximum superoxide ($O_2^{\cdot-}$) radical scavenging activity was 56.45% at 60 µg/mL concentration (Table 4 and Figure 4) and the IC_{50} was 46.28 µg/mL concentration. It was compared with the standard ascorbic acid (IC_{50}=31.18 µg/mL concentration).

Table 4: Superoxide ($O_2^{\cdot-}$) radical scavenging activity of methanol bark extract of *A. lebbeck*

S. No	Concentration (µg/mL)	% of inhibition*
1	10	16.38±0.15
2	20	24.74±0.38
3	30	31.71±0.22
4	40	43.21±0.29
5	50	52.26±0.26
6	60	56.45±0.35

(*Average value of 3 replicates)

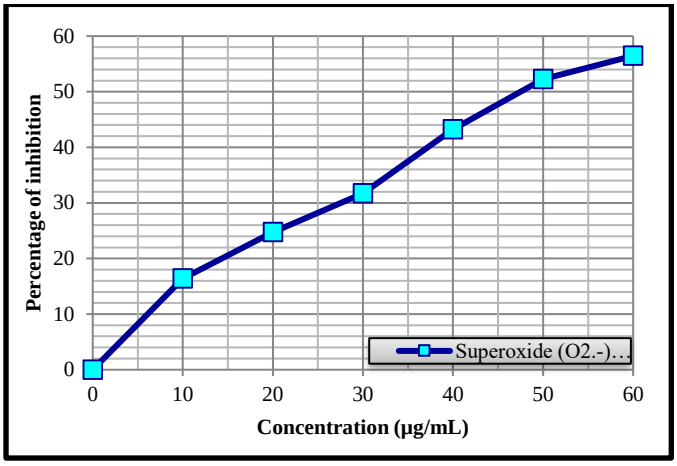


Figure 4: Superoxide ($O_2^{\cdot-}$) radical scavenging activity of methanol bark extract of *A. lebbeck*

Phosphomolybdenum reduction activity

The total antioxidant activity of methanol bark extract of *A. lebbeck* was measured by phophomolybdenum reduction method which depends on the reduction of Mo (VI) to Mo (V) by the green phosphate/Mo (V) complex at acidic pH, with a maximum absorption at 695 nm. The maximum phosphomolybdenum reduction was 88.98% at 120 µg/mL concentration and the RC_{50} was 31.39 µg/mL concentration (Table 5 and Figure 5). It was compared with the standard ascorbic acid (RC_{50} = 5.97 µg/mL concentration).

Ferric (Fe^{3+}) reducing power activity

The reducing power activity was assessed based on the reduction of $Fe^{3+}$to Fe^{2+} by the methanol bark extract of *A. lebbeck* and the subsequent formation of ferro-ferric complex. The reducing ability increases with increasing concentration of the extract. The maximum Fe^{3+} reduction was 80.55% at 120 µg/mL concentration and the RC_{50} was 39.16 µg/mL concentration (Table 5 and Figure 5). It was compared with the standard ascorbic acid (RC_{50} = 29.11 µg/mL concentration).

Table 5: Phosphomolybdenum reduction and Ferric (Fe^{3+}) reducing power activity of methanol bark extract of *A.lebbeck*

S. No	Concentration (µg/mL)	% of reduction*	
		Phosphomolybdenum reduction	Fe^{3+} reduction
1	20	37.50±0.23	26.62±0.37
2	40	63.71±0.46	51.07±0.19
3	60	74.86±0.24	68.45±0.45
4	80	83.58±0.15	75.00±0.25
5	100	84.64±0.32	78.57±0.40
6	120	88.98±0.28	80.55±0.34

(*Average value of 3 replicates)

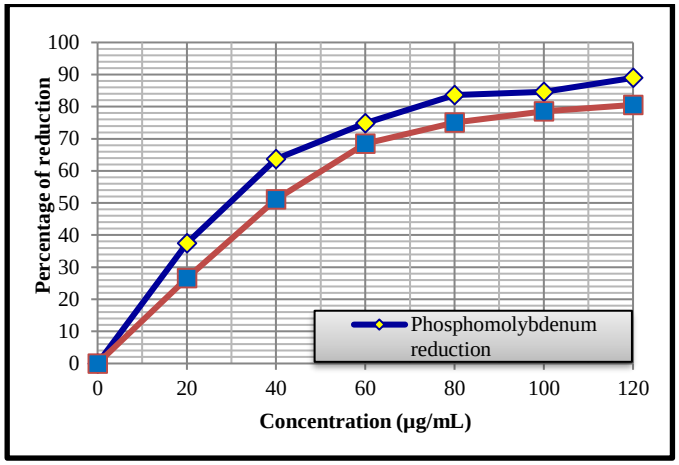


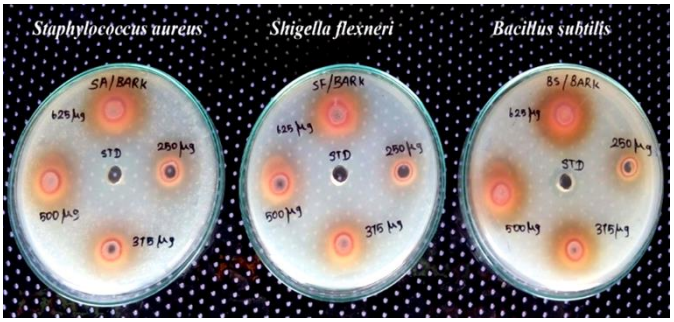
Figure 5: Phosphomolybdenum reduction and Ferric (Fe^{3+}) reducing power activity of methanol bark extract of *A.lebbeck*

Antibacterial activity by agar well diffusion method

The methanol bark extract of *A. lebbeck* was investigated for antimicrobial activity against microorganisms including *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Shigella flexneri.* The antimicrobial sensitivity of the crude extract and their potency were assessed quantitatively by measuring the diameter of clear zone in cultures in petri-plates (Table 6 and Figure 6). The antimicrobial activity of these extracts could be correlated by the availability of phytochemicals such as flavonoids, phenolic compounds, terpenoids, tannin and alkaloids that adversely affect the growth and metabolism of microbes. The comparatively poor antimicrobial profile indicated by the bark extract which contain lesser levels of alkaloids and terpenoids, as evident from the quantitative analysis supports this correlation.

Table 6: Antibacterial activity of methanol bark extract of *A. lebbeck*

S. No	Organisms	Zone of inhibition (mm)				Standard (Tetracycline)
		250 µg	375 µg	500 µg	625 µg	
1	*Staphylococcus aureus*	13	16	20	24	20
2	*Shigella flexneri*	10	12	16	20	22
3	*Bacillus subtilis*	12	15	18	23	26
4	*Escherichia coli*	12	15	18	22	28
5	*Proteus vulgaris*	10	13	20	24	20
6	*Klebsiella pneumoniae*	10	15	20	22	24



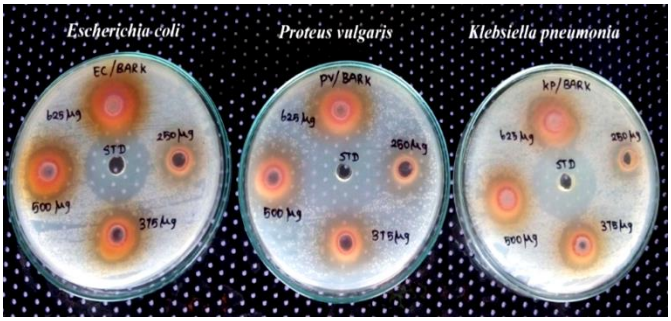


Figure 6: Antibacterial activity of methanol bark extract of *A. lebbeck*

The maximum antibacterial effect of methanol bark extract of *A. lebbeck* was found to be 24 mm against *Staphylococcus aureus* and *Proteus vulgaris* at 625 µg concentration respectively by agar well diffusion method.

Thin layer chromatography

Thin layer chromatography analysis was evaluated for the selected solvent system of toluene: ethyl acetate with the ratio of 1:1. The Rf value calculated was found to be 0.92, 0.74, 0.46 and 0.32 (Figure 7).

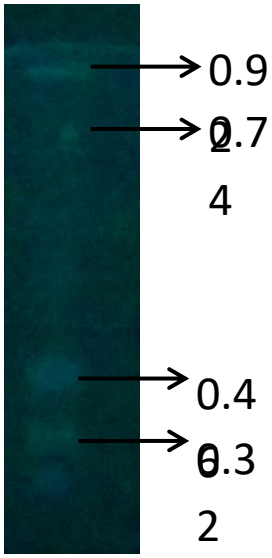


Figure 7: Active Compounds separated by Thin layer chromatography

Anticancer activity of methanol bark extract of *A. lebbeck* on MCF 7 (Human breast cancer) cell lines

Cancer chemoprevention with natural phytochemical compounds is an emerging strategy to prevent, delay or cure cancer. The MTT assay is a colorimetric method for cell metabolic assessment. The MTT *in vitro* cell proliferation method is one of the most widely used assays for evaluating preliminary anticancer activity of synthetic derivatives, natural products and natural product extracts. This assay gives an indication of whole cell cytotoxicity; however, to determine the exact molecular target to be perform kinase enzyme activity that play a key role in several physiological processes and their inhibitors have been found to exhibit anticancer activity against various human cancer cell lines. The MTT assay involves the conversion of the water soluble MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to an insoluble formazan. The formazan is then solubilized, and the concentration was determined by optical density at 570 nm. The maximum MCF 7 cell death was 79.36% at 250 µg/mL concentration (Table 7 and Figure 8) and the IC_{50} was 47.12 µg/mL concentration.

Table 7: Cytotoxic activity of methanol bark extract of *A. lebbeck* on MCF 7 (Human breast cancer) cell lines

S. No	Concentration (µg/mL)	% of cell death
1	0.781	2.79±0.20
2	1.562	3.91±0.36
3	3.125	3.12±0.35
4	6.25	9.60±0.10
5	12.5	36.26±0.47
6	25	42.31±0.14
7	50	53.06±0.26
8	100	68.61±0.19
9	250	79.36±0.23

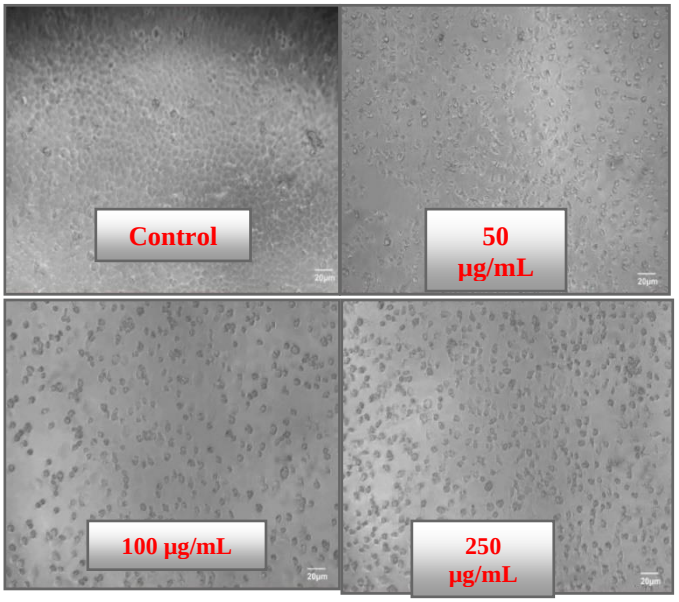
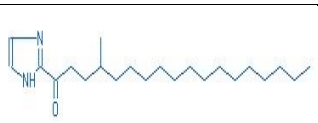
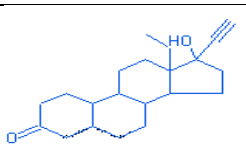
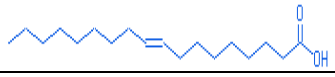
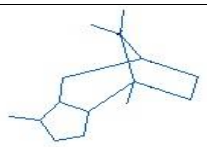
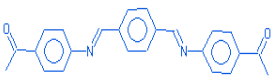
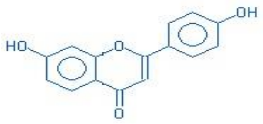


Figure 8: Cytotoxic activity of methanol bark extract of *A. lebbeck* on MCF 7 (Human breast cancer) cell lines

Gas Chromatography–Mass Spectrometry analysis

GC-MS analysis was performed for the methanol bark extract of *A. lebbeck* and the eluted compounds were showed in Table 8 and Figure 9. Levonorgestrel (Levo-5a-dihydronorgestrel) is used in emergency contraceptive pills (ECPs), both in a combined Yuzpe regimen which includes estrogen. Elaidic acid (Octadec-9-enoic acid) occurs naturally in small amounts in caprine and bovine milk (very roughly 0.1% of the fatty acids) and in some meats. Elaidic acid increases plasma cholesteryl ester transfer protein (CETP) activity which lowers HDL cholesterol. Liquiritigenin is an flavanone and is an estrogenic compound which acts as a selective agonist of the ERβ subtype of the estrogen receptor (ER). Levonorgestrel is combined with estradiol in estrogen patch for menopausal hormone therapy.

Table 8: Active compounds eluted and identified in methanol bark extract of *A.lebbeck* by GCMS analysis

S. No	RT	Compound Name	Compound Structure	Mol. Wt (g/mol)	Mol. Formula
1	23.55	2-(4-Methyloctadecanoyl)imidazole		348.566	$C_{22}H_{40}$
2	23.22	Levo-5a-dihydronorgestrel		314.46	$C_{21}H_{30}O_2$
3	21.18	9-Octadecyonoic acid, methyl ester		294.47	$C_{19}H_{34}O_2$
4	19.2	Octadec-9-enoic acid		282.46	$C_{18}H_{34}O_2$
5	18.53	10-Octadecenoic acid,methyl ester		296.49	$C_{19}H_{36}O_2$
6	16.95	Dodecanoic acid,11-oxo-methyl ester		228.32	$C_{13}H_{24}O_3$
7	15.85	4,7-Methanoazulene,decahydro-1,4,9,9-tetramethyl		206.37	$C_{15}H_{26}$
8	12.05	Benzene,1-pentynyl		144.21	$C_{11}H_{12}$
9	26.93	Benzene,1,4-bis(4-acetylphenyliminomethyl)		368.428	$C_{24}H_{20}N_2O_2$
10	17.58	4H-1 Benzopyron-4-one,7-hydroxy-2-(4-hydroxy phenyl)Liquiritigenin		256.25	$C_{15}H_{12}O_4$

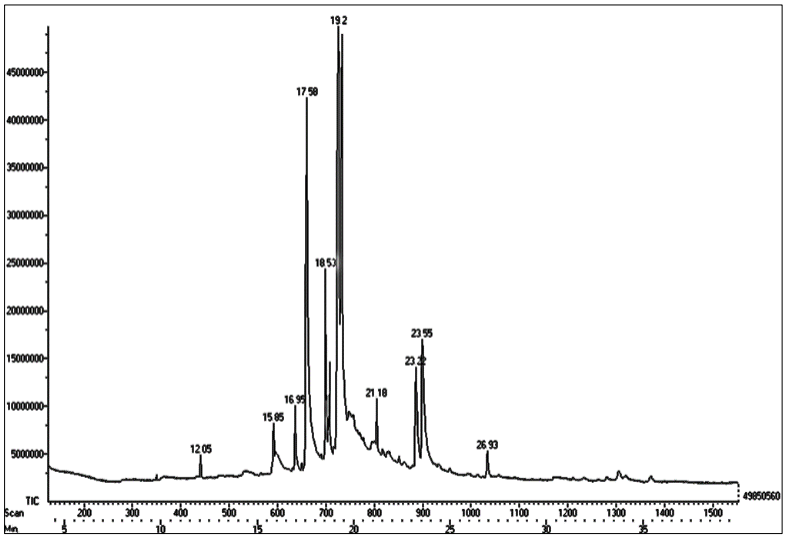


Figure 9: GCMS Chromatogram of methanol bark extract of *A. lebbeck*

CONCLUSION

The results of the research work indicate that methanol bark extract of *A. lebbeck* has significant antioxidant activities to reduce harmful effect of radicals. Further molecular studies, animal studies and clinical studies are required to find out the mechanism of action of bioactive compounds present in *A. lebbeck* before it can be recommended for any practical widespread use of the plant. The results of various experiments conducted in the present study provide promising guideline regarding the potential uses of *A. lebbeck* as an antioxidant agent. In addition, in the development of medicine from medicinal plants, among other things, a practical plan should be developed to preserve these resources.

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REFERENCES

1. Little EL Jr. Common fuel wood crops. A handbook for their identification. 1983; Morgantown, WV: Commune-Tech Associates.
2. Doran JC, Turnbull JW. (eds.). Australian trees and shrubs: species for land rehabilitation and farm planting. 1997; ACIAR Monograph No.24. Canberra, Australia, ACIAR. 384p.
3. Lowry JB, Prinsen JH, Burrows DM. *Albizia lebbeck* - a promising forage tree for semiarid regions. Forage tree legumes in tropical agriculture, 1994; 24:75-83.
4. Davis WL, Matthew SB. Antioxidants and cancer III: quercetin, Alternative Medicine Review. 2000; 5:196-208.
5. Nielsen I. The Malesian species of *Acacia* and *Albizia* (Leguminosae-Mimosoideae). Opera Botanica. 1985; 81:1-50.
6. Hyland BPM, Whiffin T. Australian tropical rain forest trees. An interactive identification system, 1993; 2. CSIRO, Australia.
7. Harbome JB. Phytochemical Methods. Chapman and Hall Ltd. London, 1973; 49-188.
8. Raaman N. Phytochemical techniques. New India Publishing Agency. New Delhi. 2006; 306.
9. Spanos GA, Wrosltad RE. Influence of processing and storage on the phenolic composition of Thompson seedless grape juice. Journal of Agricultural & Food Chemistry. 1990; 38:1565-1571.
10. Liu X, Dong M, Chen X, Jiang M, Lv X, Yan G. Antioxidant activity and phenolics of endophytic *Xylaria* sp. from *Ginkgo biloba*. Food Chemistry, 2007; 105:548-554.
11. Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoids content in propolis by two complementary colorimetric methods. J. Food Drug Analysis. 2002; 10:178-182.
12. Brand-Williams W, Cuvelier ME, Berset C. Use of free radical method to evaluate antioxidant activity. Lebensmittel Wissenschaft and Technologie, 1995; 28:25-30.
13. Ravisankar N, Sivaraj C, Seeni S, Joseph J, Raaman N. Antioxidant activities and phytochemical analysis of methanol extract of leaves of *Hypericum hookerianum.* Int. J. Pharm. Pharm. Sci. 2014; 6:456-460.
14. Prieto P, Pineda M, Anguilar M. 'Spectrophotometric quantisation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific application to the determination of Vitamin E. Anal. Biochem, 1999; 269:337-34.
15. Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. Journal of Agricultural and Food Chemistry. 1995; 43:27-32.
16. Saowapa R, Soottwat B, Thummanoon P. Extraction antioxidative, and antimicrobial activities of brown seaweed extracts, *Turbinaria ornata* and *Sargassum polycystum*, grown in Thailand. International aquatic research. 2015; 7(1):1-16.
17. Sheyin Z, Maimako J, Shindang J, Essien CU, Bigwan EI, Ede FR. Antimicrobial activity of *Albizia lebbeck* leaf extract on some medically important bacteria. International journal of current microbiology and applied sciences. 2015; 4(9):473-477.
18. Stahl E. Thin Layer Chromatography. 2nd ed. *Springer Pvt Ltd*. New Delhi, 2005; 85.
19. Mossman T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J Immunol Methods, 1983; 65:55-63.
20. Sivaraj C, Aswitha V, Srinidhi M, Saraswathi K, Arumugam P. Antibacterial, antioxidant activities and GC-MS analysis of leaves extract of *Millingtonia hortensis* L. The Pharma Innovation Journal. 2019; 8(1):513-521.
21. Nakamura Y, Watanabe S, Miyake N, Kohno H, Osawa T. Dihydrochalcones: Evaluation as Novel Radical Scavenging Antioxidants. Journal of Agricultural and Food Chemistry. 2003; 51:3309-3312.
22. Yen GC, Duh PD. Scavenging effect of methanolic extracts of peanut hulls on free-radical and active-oxygen species. Journal of Agricultural and Food Chemistry. 1994; 42(3):629-632.
23. Liu F, Ooi VEC, Chang ST. Free radical scavenging activities of mushroom polysaccharide extracts. Life Sciences, 1997; 60(10):763-771.

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